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Article

Amazonian Fruit Mari-Mari (*Cassia leiandra* Benth.): Identification of Flavonoids and Antioxidant, Antimicrobial and Antiproliferative Properties

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Mari-mari (*Cassia leiandra* Benth.) is an Amazonian native fruit that is highly appreciated in the northern region of Brazil, but there are few studies regarding its chemical composition and/or biological properties. A phytochemical study was conducted on the extracts of the pulp, peel and seeds; and their antioxidant, cytotoxic and antimicrobial potential were also determined. Methanol and acetone extracts from different fruit parts were subjected to total phenolic, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Fe^{III} reducing capacity (FRAP) assays. From the peel extracts, the engeletin and astilbin could also be isolated and characterized based on nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry. A quantification using ultra-fast liquid chromatography of acetone dry crude extracts (DCE) of the peel showed that engeletin was the main compound (532.77 mg g^{-1} DCE). Astilbin content was also determined (131.53 mg g^{-1} DCE). The antibacterial and antiproliferative activities were evaluated and the methanol extract of the peel showed higher growth inhibition activity against all tested bacteria. Engeletin showed a relevant antimicrobial effect against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*. Acetone and methanol peel extracts exhibited a relevant inhibitory effect on the proliferation of breast carcinoma cells (MCF-7), with no toxicity to the normal cell line (NIH/3T3).

Keywords: *Cassia leiandra*, mari-mari, total phenolic contents, antioxidant property, cytotoxic activity, antimicrobial activity

Introduction

The Amazon rainforest has a wide variety of plant species that have important nutritional properties, and many of these properties are associated with small molecules present in their composition.[1,2] A variety of edible fruit species from the Amazon region have been reported as a source of antioxidant compounds, especially polyphenols, which are a well-known class of metabolites with different biological activities, including antioxidant and antiproliferative activities.[3-5]

Cassia leiandra (Fabaceae) is native to the Brazilian Amazon, where it is known as mari-mari, "*mari*", "*ingá-mari*", and "*seruaia*". Its fruits have the shape of a pod (yellowish when ripe), cylindrical, 40-50 cm long, and 3.0-4.0 cm in diameter, whose seeds are wrapped in succulent and pasty pulp, with a bittersweet flavor that is

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similar to the taste of tamarind (*Tamarindus indica*). It is usually consumed raw by the local population and is often found in street markets in the Amazon. The fruiting period occurs when the river level is high (between January and May), and fruit production can reach 1,836 kg ha^{-1}.[6]

For the genus *Cassia*, there are many chemical studies related to different parts (roots, stems, bark, seeds, and leaves) and these report the presence of glycosides, flavonoids, and derivatives of anthracene. Other studies have reported hepatoprotective, anti-inflammatory, antimutagenic, anti-ulcer, antibacterial, antifungal and antioxidant properties in extracts obtained from different *Cassia* species.[7-9]

Despite numerous reports about the genus *Cassia*, the chemical composition of *C. leiandra* fruits has not yet been studied. To date, there are no reports on the biological properties and/or antioxidant, antimicrobial and antiproliferative potential of the fruits of this species. In this work, the antioxidant potential of acetone and methanol extracts obtained from the pulp, peel, and seeds were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and the ferric reducing antioxidant power (FRAP) assay. The total phenolic content was determined using the Folin-Ciocalteu method. In addition, the flavonoids engeletin and astilbin were isolated using high-performance liquid chromatography (HPLC), and were identified using 1D and 2D nuclear magnetic resonance (NMR), and quantified using ultra-fast liquid chromatography (UFLC). Considering the potential applications of natural antioxidants in food and nutraceutical technology, our results aim to contribute to the phytochemical knowledge and technological applications of a promising edible fruit from the Amazon.

Experimental

Chemicals

Acetone and methanol (P.A.), methanol and acetonitrile (ACN) HPLC grade ultrapure water, formic acid, dimethyl sulfoxide-d_6 (deuterated DMSO), methanol-d_4 (deuterated methanol), astilbin from *Engelhardtia roxburghiana* ≥ 98%, Folin Ciocateu reagent, formic acid, gallic acid, quercetin, sodium carbonate (Na_2CO_3), iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), sulforhodamine B (SRB), Trizma® base buffer and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Ltd. (Saint Louis, USA). Breast cancer cell MCF-7 and murine fibroblast NIH/3T3 cell lines, fetal bovine serum (FBS, Gibco, Thermo Fisher, USA), penicillin, and streptomycin were also purchased from Sigma-Aldrich Ltd. (Saint Louis, USA). The MCF-7 cell line used was donated by Centro de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), Universidade Estadual de Campinas, Brazil. Fresh, ripened mari-mari fruits were acquired at the local market in the Jorge Teixeira district in Manaus, Amazonas, Brazil in April 2013. All fruits (9 kg) were manually separated into pulp, peel and seeds.

Preparation of *C. leiandra* extracts

The fresh pulps, peels and seeds of the mari-mari were crushed and subjected to extraction in acetone followed by extraction in methanol in an ultrasonic bath, (Unique, USC 2800, Brazil), for 40 min at room temperature (25 °C). The dry crude extracts (DCE) were vacuum filtered and then transferred to amber flasks and stored at −36 ºC before analysis.[10,11]

Isolation of flavonoids

The powdered mari-mari peel (360.0 g) was subjected to maceration in acetone at room temperature for 48 h. During concentration under vacuum, a white precipitate (6.75 g) was obtained in a large quantity that was cold-washed with acetone, filtered and finally purified by preparative HPLC configured with Shimadzu LC-6AD pumps, a SPD-10AV UV detector and a Rheodyne injector. A Luna C18 column (Phenomenex, 250 mm × 4.6 mm, 100 Å, 5.0 μm) was used with isocratic elution with CH_3OH:H_2O (45:55 v/v) as the mobile phase at a flow rate of 6 mL min^{-1} to yield compounds **1** (13.8 mg) and **2** (60.7 mg).

Nuclear magnetic resonance (NMR) experiments

One (1D) and two-dimensional (2D) NMR spectra of astilbin **1**, in DMSO-d_6, and engeletin **2**, in methanol-d_4, were acquired on a 300 MHz spectrometer (Bruker Fourier, Billerica, USA) (300.20/75.0 MHz for $^1H/^{13}C$ NMR), and on a 600 MHz spectrometer (Bruker Avance III Billerica, USA) (600.13/125.0 for $^1H/^{13}C$ NMR).

Astilbin, dihydroquercetin 3-rhamnoside, ($C_{21}H_{22}O_{11}$) **1** white powder; UV-Vis (methanol) λ_{max} / nm 299; ^1H NMR (500 MHz, DMSO-d_6) δ 5.23 (1H, d, *J* 9.9 Hz, H-2), 4.64 (1H, d, *J* 9.9 Hz, H-3), 5.89 (1H, d, *J* 2.1 Hz, H-6), 5.90 (1H, d, *J* 2.1 Hz, H-8), 6.87 (1H, s, H-2'), 6.72 (1H, d, *J* 8.6 Hz, H-5'), 6.79 (1H, d, *J* 8.4 Hz, H-6'), 4.01 (1H, d, *J* 1.2 Hz, H-1"), 3.93 (1H, m, H-2"), 3.14 (1H, m, H-3"), 3.08 (1H, m, H-4"), 2.49 (1H, m, H-5"), 1.04 (3H, d, *J* 6.0 Hz, H-6"), 9.00 (1H, s, OH-3'), 9.10 (1H, s, OH-4'), 11.82 (1H, s, OH-5), 11.79 (1H, s, OH-7); HRMS (ESI) *m/z*, calcd. for $C_{21}H_{22}O_{11}$ $[M - H]^-$: 449.1089, found: 449.0773.

Engeletin, dihydrokaempferol 3-rhamnoside ($C_{21}H_{22}O_{10}$) **2** white powder; UV-Vis (methanol) λ_{max} / nm 298; ^{1}H NMR (600 MHz, methanol-d_4) δ 5.14 (1H, d, *J* 10.7 Hz, H-2), 4.61 (1H, d, *J* 10.7 Hz, H-3), 5.89 (1H, d, *J* 2.1 Hz, H-6), 5.90 (1H, d, *J* 2.1 Hz, H-8), 7.35 (2H, d, *J* 8.6 Hz, H-2' and H-6'), 6.84 (2H, d, *J* 8.6 Hz, H-3' and H-5'), 4.01 (1H, d, *J* 1.5 Hz, H1"), 3.50 (1H, m, H-2"), 3.64 (1H, m, H-3"), 3.30 (1H, m, H-4"), 4.24 (1H, m, H-5"), 1.18 (3H, d, *J* 6.3 Hz, H-6); HRMS (ESI) *m/z*, calcd. for $C_{21}H_{22}O_{10}$ $[M - H]^-$: 433.1140, found: 433.1100.

Quantitative analysis of isolated compounds

The engeletin isolated from the mari-mari peel was used as a standard to prepare solutions in the range from 0.48 to 500 μg mL^{-1} in ACN:H_2O (1:1 v/v). Commercial astilbin from *Engelhardtia roxburghiana* (purity ≥ 98%) from Sigma (Saint Louis, USA) was used to prepare solutions in the range of 0.29 to 150 μg mL^{-1} also using ACN:H_2O (1:1 v/v) as the solvent. These solutions were prepared fresh on different days to obtain the calibration curves. Mari-mari extracts were diluted in ACN:H_2O (1:1 v/v) in the following concentrations: methanol extract of peel (350 μg mL^{-1}), acetone extract of the peel (250 μg mL^{-1}), methanol extract of the seeds (5,000 μg mL^{-1}), methanol extract of the pulp (5,000 μg mL^{-1}). The samples were previously filtered through a 0.2 μm polytetrafluoroethylene (PTFE) membrane and analyzed using UFLC. Quantification by chromatography was performed using a chromatograph (UFLC Shimadzu Prominence, Kyoto, Japan), with a binary pump (LC-20AD), automatic injector (SIL-20A HT), column oven (CTO-20AC), diode array detection system (SPD-M20A) and chromatography data station software (LC-Solution). The column used was a C18 analytical column (Kinetex, 100 × 2.1 mm, 2.6 μm particle) was used at 30 °C. A linear gradient with 0.1% aqueous solution of formic acid (eluent A) and acetonitrile with 0.1% of formic acid (eluent B) were used as mobile phases. The elution started with 15% eluent B for 22 min, increasing linearly to 100% B during 3 min and holding constant at 100% B for 3 min. The column was reconditioned to 15% B for 7 min before analysis. The injection volume was 1 μL and the mobile phase flow was 0.2 mL min^{-1}. The concentration of astilbin and engeletin were expressed as mg of compound *per* g of DCE.

Total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu method,[12,13] in which 1.0 μL of each sample and 1 mL of Folin-Ciocalteu reagent (diluted 10-fold) were mixed in a vortex mixer, incubated for 5 min at room temperature, and then 1.0 mL of 10% Na_2CO_3 solution was added. The mixture was incubated for 90 min, and the absorbance was determined at 725 nm. The TPC was expressed as mg of gallic acid equivalent (GAE) *per* g of dry crude extract (mg GAE g^{-1} DCE).

DPPH free radical scavenging assay

The free radical scavenging properties of the acetone and methanol extracts were evaluated by measuring the decrease in the absorbance of a methanol DPPH solution at 490 nm in the presence of the extract in an ELISA plate, as previously described by Yu *et al.*[14] The results were expressed as the inhibitory concentration (IC_{50}) of the extract concentration that caused 50% scavenging of radicals. Quercetin was used as a positive control (IC_{50} = 6.0 ± 5.7 μg mL^{-1}).

Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed as previously described by Benzie and Strain.[15] The results were determined according to the calibration curve with aqueous solutions of $FeSO_4$ in the range of 100-1,000 μM. The results were expressed in μM $FeSO_4$ g^{-1} DE.

In vitro antibacterial activity

The antibacterial activities of the methanol and acetone extracts were assayed against the following three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 80958), *Staphylococcus epidermidis* (ATCC 35984), and *Staphylococcus saprophyticus* (ATCC), and three Gram-negative bacteria: *Escherichia coli* (ATCC 35218), *Klebsiella oxytoca* (ATCC 13181), and *Pseudomonas aeruginosa* (ATCC 27853), using a modified microdilution method.[16] The methanol and acetone extracts obtained from the pulp, seed and peel were first dissolved in 30% DMSO (v/v) in Muller-Hinton (MH) broth to a final concentration of 1 mg mL^{-1}. Serial dilutions (in the range of 0.1 to 10 μg mL^{-1}) of each extract were prepared in sterile 96-well microtiter plates at a 200 μL final volume of MH broth. The microbial suspensions were adjusted with sterile saline to a concentration that corresponded to 0.5 on the McFarland scale (1.5 × 10^8 colony forming units (CFU) mL^{-1}). The microplates were incubated at 37 °C for 24 h, and the absorbance was read at 590 nm using a microplate reader. Commercial chloramphenicol and rifampicin were used as positive controls and DMSO solvent was used as a negative control. The results were presented as minimum inhibitory concentration (MIC) values, which were defined

as the lowest concentration of the extract (in μg mL^{-1}) that inhibits the bacterial growth when compared to the control. The antibacterial activity of the isolated engeletin was also evaluated. First, the purified compound concentration was diluted in DMSO to 1 mM and, then, its effect on bacterial growth was checked in a range of 10 to 100 μM using a modified microdilution technique, according to the method described above.

Antiproliferative activity *in vitro*

For the evaluation of antiproliferative activity, one human neoplastic cell line, MCF-7 (breast) and one normal cell line, NIH/3T3 (murine fibroblast), were used. The cells were maintained in complete Roswell Park Memorial Institute (RPMI) 1640 medium that was supplemented with 10% fetal bovine serum (FBS), penicillin (10 U mL^{-1}), and streptomycin (10 μg mL^{-1}) and maintained at 37 °C under a 5% CO_2 atmosphere. The antiproliferative assay was based on colorimetric methods that used sulforhodamine B (SRB) with modifications.[17,18] The cells were seeded in 96-well plates at 7,500 cells *per* well, incubated at 37 °C under a 5% CO_2 atmosphere for 24 h and, subsequently, exposed to the test compounds dissolved in sufficient DMSO to yield concentrations of 0.025, 0.25, 2.5, and 5 μg mL^{-1}, and then reincubated for another 48 h. The final concentrations of DMSO did not affect cell viability. After incubation, the supernatant was removed, and the cells were fixed in 100 μL of trichloroacetic acid (20%). After 30 min, they were stained with 50 μL of 0.1% SRB dissolved in 1% acetic acid. Excess dye was removed with 1% acetic acid. Finally, the proteins were solubilized in Trizma® base buffer (10 mM, pH = 10.5) and readings were performed at 540 nm on a microplate reader. The positive control was doxorubicin at concentrations of 0.025, 0.25, 2.5, and 25 μg mL^{-1}. The results were expressed as the inhibition of 50% growth (IC_{50}).

Statistical analysis

All experiments were performed in triplicate, and the data were expressed as the mean ± standard deviation (SD). The IC_{50} values were determined via interpolation.

Results and Discussion

Structural identification of isolated flavonoids

The semi-preparative HPLC of acetone extract of the peel led to the isolation of flavonoids **1** and **2** (Figure 1). Based on ^1H and ^{13}C NMR and HRMS analyses, the isolated compounds were identified as the flavonoids astilbin **1** and engeletin **2**, which have been previously reported in the literature, though this is the first time in *C. leiandra*.[19-21] Astilbin (Figure 1) was isolated as a white powder and the molecular formula $C_{21}H_{22}O_{11}$ was established using HRMS (experimental *m/z* 449.0773, calculated *m/z* 449.1089). The ^1H NMR spectrum of astilbin shows the two doublets δ 5.89 and 5.90 (*J* 2.1 Hz), which are characteristic of hydrogens H-8 and H-6 of oxygenated flavanones in positions 5 and 7. One doublet at δ 6.79 (*J* 8.6 Hz), one doublet at δ 6.72 (*J* 8.4 Hz), and one singlet at δ 6.87 were assigned to the hydrogens H-6', H-5' and H-2', respectively from ring B. Additionally, this spectrum showed one doublet at δ 4.01, corresponding to anomeric hydrogen, with multiplets between δ 2.49-3.93 and one doublet at δ 1.04. These data were consistent with the rhamnose unit. The coupling constant *J* 1.5 Hz observed in the ^1H NMR spectrum corroborates the rhamnoside unit. The correlation observed in the heteronuclear multiple bond correlation (HMBC) of δ 4.01 (H-1") with δ 102.4 confirmed the unit rhamnose at C-3. After analysis of the spectral data and comparisons with data described in the literature, it was possible to identify compound **1** as dihydroquercetin 3-rhamnoside (astilbin).[19]

Similarly, compound **2**, which was isolated as a white powder, was identified as engeletin (Figure 1). The molecular formula $C_{21}H_{22}O_{10}$ was established using HRMS (experimental: *m/z* 433.1100 calculated: *m/z* 433.1140). The ^1H NMR spectrum of **2** was quite similar to spectrum of **1** (a flavanone), except for the appearance of two doublets, both integrated for two hydrogens (*J* 8.6 Hz) at δ 7.35 (H-2'/H-6') and 6.85 (H-3'/H-5'), corresponding a disubstituted benzene ring, which has hydroxyl in position 4. The comparison of ^1H and ^{13}C NMR spectra with literature data led to the identification of the compound **2** as engeletin (dihydrokaempferol 3-rhamnoside) (Figure 1), which is a glycosylated flavonoid, also reported in other genera of Fabaceae.[19]

Total phenolic content and antioxidant activities

Table 1 shows the results of the DPPH, FRAP and TPC antioxidant activity assays of the extracts obtained from the pulp, peel, and seeds, in addition to flavonoids isolated from acetone extract of the peel. The peel extracts obtained in methanol and acetone contain twice more phenolic compounds when compared to the pulp and seed extracts that were extracted using an ultrasonic bath. According to Ayala-Zavala *et al.*,[22] the contents of the functional compounds in different tropical exotic fruits are most often located in the peel and seeds, with lower concentrations in the pulp.

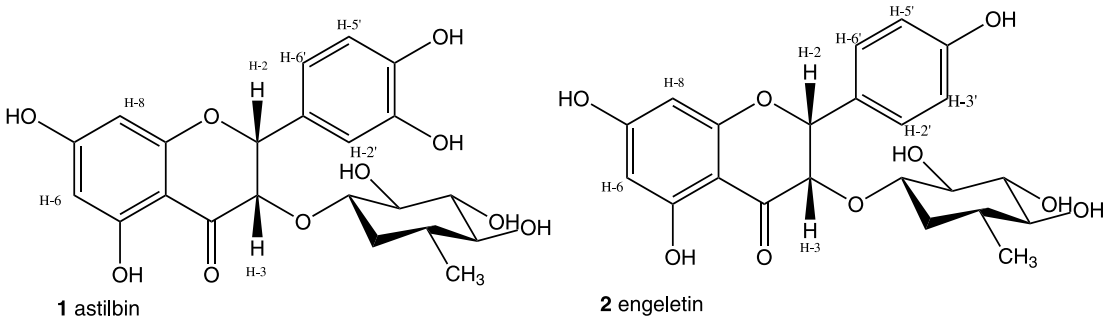

Figure 1. Structure of compounds **1** (astilbin) and **2** (engeletin) isolated from mari-mari fruit.

These results are in agreement with the FRAP assay results, since methanol and acetone extracts of the peel (1,240.71 ± 0.06 and 1,624.46 ± 0.09 μmol $FeSO_4$ g^{-1} dry crude extract (DCE), respectively), showed higher ferric-reducing ability. The values obtained for the acetone extract of peel in the DPPH assay (IC_{50} = 199.44 ± 0.01 μg mL^{-1} m v^{-1}) confirmed the presence of compounds with greater potential for scavenging free radicals when compared to the other extracts (pulp and seed in acetone). Considering the extracting solvent, the methanol extracts of pulp, peel and seed showed relatively similar results, but these were slightly inferior to the acetone extracts. The DPPH results obtained for astilbin and engeletin (IC_{50} = 316.66 ± 0.06 and 3,710.09 ± 0.005 μg mL^{-1}, respectively) show that engeletin cannot scavenge DPPH radicals. Engeletin probably does not contribute to the antioxidant activity of the peel extracts, whereas astilbin, which presented moderate capacity, may contribute to the antioxidant activities in the peel extracts.

Quantification of astilbin and engeletin

The calibration curves obtained from engeletin and astilbin using UFLC presented good linearity (R^2 = 0.99) for both substances. The quantification using UFLC showed that these flavonoids are abundant in the acetone extracts of the peel at concentrations of 532.77 and 131.53 mg g^{-1} DCE for engeletin (retention time of 14.1 min) and astilbin (retention time of 8.0 min), respectively (Table 2). These data corroborate the GAE values in the Folin-Ciocalteu assays for this extract, which stood out for having the highest total phenolic content.

In vitro antibacterial activity

Table 3 shows the results of antibacterial activities of the extracts obtained from *C. leiandra* (mari-mari) fruit. The evaluated bacterial responses varied among different extracts, but the methanol extract of the peel presented better inhibitory activity against the growth of all the tested Gram-positive and Gram-negative bacteria and resulted in a MIC range of 25 to 35 μg mL^{-1}. Meanwhile, the acetone extract of the peel revealed poor antibacterial activity (MIC > 40 μg mL^{-1}). On the other hand, the methanol and acetone extracts of the pulp (MIC > 100 μg mL^{-1}) and seeds (MIC was not assayed at the tested concentrations) did not show any antibacterial effect. The methanol extract of the peel was relatively more effective towards Gram-positive pathogenic bacteria. These results are in accordance with previous studies,[23] which indicate that Gram-negative bacteria are, in general, more resistant to antimicrobial

Table 1. Antioxidant activity of sample extracts and isolated flavonoids of mari-mari

Sample	TP[a] / (mg GAE g^{-1})	DPPH IC_{50}[a] / (μg mL^{-1} m v^{-1})	FRAP[a] / (μmol $FeSO_4$ g^{-1} DCE)
Pulp (methanol)	39.97 ± 0.019	620.30 ± 0.01	629.88 ± 0.02
Peel (methanol)	119.90 ± 0.019	619.14 ± 0.04	1,240.71 ± 0.06
Seeds (methanol)	39.24 ± 0.032	673.73 ± 0.01	406.96 ± 0.09
Pulp (acetone)	65.14 ± 0.040	364.48 ± 0.001	634.46 ± 0.03
Peel (acetone)	117.69± 0.009	199.44 ± 0.01	1,624.46 ± 0.09
Seeds (acetone)	60.17 ± 0.005	456.43 ± 0.02	466.12 ± 0.02
Engeletin	–	3,710.09 ± 0.005	190.13 ± 0.002
Astilbin	–	316.66 ± 0.06	1,905.71 ± 0.07
Quercetin	–	5.7 ± 0.02	2774.88 ± 0.15

[a]Values are shown as mean ± standard deviation (n = 3). TP: total phenolic; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: half maximal inhibitory concentration; FRAP: ferric ion reducing antioxidant power; GAE: gallic acid equivalent; DCE: dry crude extract.

Table 2. Content of engeletin and astilbin in the dry extracts (DE) of mari-mari fruits

Extract	Content / ($mg_{engeletin}$ *per* g_{DE})	CV / %	Content / ($mg_{astilbin}$ *per* g_{DE})	CV / %
Peel (acetone)	532.77	5.8	131.53	5.72
Peel (methanol)	167.76	4.7	46.55	4.10
Seeds (methanol)	2.46	1.7	2.06	1.86
Pulp (methanol)	0.14	0.8	0.19	0.46
LOD / (mg mL^{-1})	0.48		0.29	
LOQ / (mg mL^{-1})	1.95		1.17	

CV: coefficient of variation; LOD: limit of detection; LOQ: limit of quantification.

Table 3. Antibacterial activity of the mari-mari fruit extracts

Sample	MIC / (μg mL^{-1})					
	Gram-positive bacteria			Gram-negative bacteria		
	S. aureus (ATCC 80958)	*S. epidermidis* (ATCC 35984)	*S. saprophyticcus* (ATCC 49453)	*E. coli* (ATCC 35218)	*K. oxytoca* (ATCC 13181)	*P. aeruginosa* (ATCC 27853)
Methanol extract						
Pulp	> 100	> 100	> 100	> 100	> 100	> 100
Seed	NA	NA	NA	NA	NA	NA
Peel	25	25	20	30	25	35
Acetone extract						
Pulp	> 100	> 100	> 100	> 100	> 100	> 100
Seed	NA	NA	NA	NA	NA	NA
Peel	> 40	> 40	> 40	> 40	> 40	> 40
Standards						
Chloramphenicol	7.2	3.9	3.2	3.9	4.2	12.2
Rifampicin	0.8	0.8	0.8	2.2	1.2	10

NA: not assayed at tested concentrations; MIC: minimum inhibitory concentration.

agents than Gram-positive microorganisms, and this is probably due to the presence of an outer-membrane permeability barrier in these cells. Furthermore, engeletin, a glycosilated flavonoid isolated from the mari-mari peel, showed promising activity against *S. aureus*, *S. epidermidis*, and *E. coli* with MICs of 50 μM.

In vitro antiproliferative activity

Flavonoids are known to be synthesized by plants in response to microbial infections;[24] therefore, it is expected that they have effective *in vitro* antibacterial activity. Flavonoid-rich plant extracts from different species have been reported to present an antimicrobial effect.[24,25] In addition, Mori *et al.*[26] have suggested that the flavonoids may intercalate or form hydrogen bonds with the stacking of nucleic acid bases due to their B ring configuration and further lead to the inhibition of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis in bacteria. The inhibitory activities of quercetin, apigenin, and 3,6,7,3',4'-pentahydroxyflavone against *Escherichia coli* DNA gyrase were defined by Ohemeng *et al.*[27] Regarding the evaluation of the *in vitro* antiproliferative activity (Table 4), the acetone and methanol extracts of the peel exhibited a relevant inhibitory effect on breast carcinoma cell proliferation, with efficiency concentration (EC_{50}) values of 5.43 and 8.94 μg mL^{-1}, respectively. Furthermore, the extracts of pulp and seeds did not present inhibition of growth activity against the human cancer cell line (MCF-7). The growth inhibition assay showed that non-tumor cells (NIH/3T3 line) presented a higher EC_{50} (> 100 μg mL^{-1}) when compared to the human tumor line (MCF-7) at the same exposure time, which indicates lower cytotoxicity. These results indicate that mari-mari fruit peels should be consumed, instead of being discarded.

Fruits with high flavonoid content, such as apples or grapes, have been reported as potential cancer chemopreventive agents.[28] Oxidative stress plays an important role in the development of chronic and degenerative ailments such as cancer, arthritis, aging, autoimmune disorders, cardiovascular, and neurodegenerative diseases.[29] Therefore, the regular consumption of fruits that are rich

Table 4. Antiproliferative effect of the mari-mari fruit extracts on cancer cell lines

Sample	EC_{50}[a] / (μg mL^{-1})	
	MCF-7[b]	NIH/3T3[c]
Methanol extract		
Pulp	>100	>100
Seed	>100	>100
Peel	8.94	>100
Acetone extract		
Pulp	>100	>100
Seed	>100	>100
Peel	5.43	>100
Positive control		
Doxorubicin	0.25	0.28

[a]Concentration necessary to inhibit 50% of the cell proliferation; [b]breast carcinoma cell line; [c]murine fibroblast cell line.

in flavonoids could minimize the cancer-promoting action of oxygen radicals.

Conclusions

The phytochemical study of the mari-mari fruit (*C. leiandra* Benth.) is described herein for the first time. Quantification of astilbin and engeletin showed that the extracts of the peel have higher concentrations of these flavonoids when compared to the seeds and pulp. Engeletin showed a relevant antimicrobial effect against *S. aureus*, *S. epidermidis* and *E. coli*. Furthermore, acetone and methanol extracts of the peel exhibited a relevant inhibitory effect on the proliferation of breast carcinoma cells (MCF-7), without presenting toxicity to the normal cell line (NIH/3T3). These results contribute significantly to the chemical knowledge and therefore demonstrate that this Amazonian fruit has potential as a nutraceutical food.

Supplementary Information

Supplementary information (^{1}H, ^{13}C, COSY, HMBC, HSQC, NOESY spectra and MS data, mari-mari fruit pulp chromatograms, analytical curve of the engeletin standard) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Mayane P. de Souza was responsible for investigation and writing - original draft; Ingrity Suelen C. Sá for investigation; Flávio Augusto de Freitas for conceptualization and writing; Afonso Duarte L. de Souza for funding acquisition; Sergio M. Nunomura for funding acquisition, validation and manuscript review; Magno P. Muniz for validation; Ana Paula R. Pereira for biological activity investigation; Bianca O.Augusto for biological activity investigation; Giovana B. Gomes for biological activity investigation; Renata T. Perdomo for experimental design and supervision; Maria Lígia R. Macedo for experimental design and data analysis; Simone S. Weber for experimental design, data analysis and manuscript writing; Rita de Cassia S. Nunomura for review and editing and supervision and funding acquisition.

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